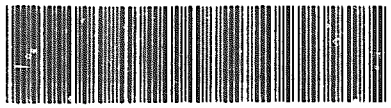


8EHQ-96-13810

Procter&Gamble

The Procter & Gamble Company
Ivorydale Technical Center
299 Spring Grove Avenue, Cincinnati, Ohio 45217-1087

Document Processing Center (TS-790)
(Attention: Section 8(e) Coordinator)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460

November 15, 1996

8EHQ-1196-13810

Contains No CBI

RE: TSCA Section 8(e) Submission
ATTN: TSCA Section 8(e) Coordinator

This submission is made in accordance with TSCA Section 8(e) requirements. We do not believe the data described in this submission reasonably support the conclusion that the subject materials present a substantial risk of injury to human health or the environment.

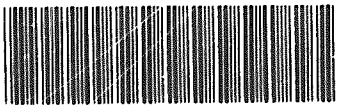
This communicates data on the effects of the oral administration of 3,6,9,12,15-Pentaoxatricosan-1-ol; CAS# !9327-40-3, to mice in an acute oral gavage toxicity study. The draft final report of the study is attached. 3,6,9,12,15-Pentaoxatricosan-1-ol was used as a reference material in this study, and we have submitted similar data for this chemical to the Agency in the past.

Pure analytical-grade 3,6,9,12,15-Pentaoxatricosan-1-ol was tested under the test substance number SS0452.01. In this test, 5 mls/kg of a 250 mM (8.76% w/w in deionized water) solution of 3,6,9,12,15-Pentaoxatricosan-1-ol was administered to ten mice (5 males and 5 females) by oral gavage. The animals were observed continuously during the first two hours after dosing and at approximately 2.5, 3.0, 3.5, 4.0, 6.0, and 8.0 hours postdose and daily thereafter for 14 days. The animals were observed and scored for the occurrence of ataxia and loss of righting reaction as indicators of neurotoxicity.

The observed symptoms were consistent with the known anesthetic effect for this material. Within two hours, 9/10 test animals which received the test article exhibited ataxia, and 5/10 exhibited impairment or loss of righting reaction. The only other behavior observed was hypoactivity in 8/10 animals. All animals fully recovered within two hours and no abnormalities were detected upon necropsy. The two other substances tested in this study did not produce any signs of neurotoxicity. Only hypoactivity was observed in a few of the test animals in these groups.

We have handled and will continue to handle this material with appropriate caution, in keeping with our standard procedures for handling all chemical substances. We will continue our practice of communicating appropriate hazard information for the test substances by both labels and MSDS.

If you wish further information, please contact me.



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RECEIVED

Very truly yours,

THE PROCTER AND GAMBLE COMPANY

W. E. Bishop, Ph. D.
Manager
Risk, Policy & Regulatory Sciences Dept.
Telephone: 513/627-6145

RECEIVED

Corning Hazleton Inc.
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96C32

CORNING Hazleton

Sponsor:

The Procter & Gamble Company
Cincinnati, Ohio

DRAFT

FINAL REPORT

Study Title:

Acute Oral Gavage Toxicity Study with SS0450.01, SS0451.01, and SS0452.01 in Mice with Additional Neurological Examinations

Author:

Steven M. Glaza

Study Completion Date:

October 11, 1996

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 60704511

Sponsor Project Identification:

SSBTS-96.010

COMPLIANCE STATEMENT

Acute Oral Gavage Toxicity Study with SS0450.01, SS0451.01, and SS0452.01 in Mice with Additional Neurological Examinations

This study was conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 792 with the exception that analysis of the prepared test material mixture for SS0452.01 for concentration, homogeneity/ solubility, and stability was not conducted.

Steven M. Glaza
Study Director
Acute Studies
Corning Hazleton Inc.

Date

Sponsor's Representative

Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Corning Hazleton Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792. The following inspections were conducted and findings reported to the Study Director and management.

Inspection Dates			Date Reported to Study	
From	To	Phase	Director	Date to Management
07/29/96	07/29/96	Protocol Review	07/29/96	07/29/96
08/20/96	08/20/96	Necropsy	08/20/96	08/20/96
09/17/96	09/17/96	Protocol Amendment	09/17/96	09/17/96
10/08/96	10/08/96	Data/Report Review	10/08/96	10/08/96

Representative, Quality Assurance Unit

Date

STUDY IDENTIFICATION

Acute Oral Gavage Toxicity Study with SS0450.01, SS0451.01, and SS0452.01 in Mice with Additional Neurological Examinations

Test Material Identification Numbers	SS0450.01, SS0451.01, and SS0452.01
Sponsor	The Procter & Gamble Company Sharon Woods Technical Center 11530 Reed Hartman Highway Cincinnati, Ohio 45217
Study Monitor	Cathy Satter, PhD The Procter & Gamble Company Sharon Woods Technical Center 11530 Reed Hartman Highway Cincinnati, Ohio 45217 (513) 626-3458 Facsimile No. (513) 626-3522
Study Director	Steven M. Glaza Corning Hazleton Inc. P.O. Box 7545 Madison, Wisconsin 53707 (608) 241-7292 Facsimile No. (608) 242-7936
Study Location	Corning Hazleton Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704
Study Timetable	
Study Initiation Date	July 30, 1996
Experimental (In-life) Start Date	August 6, 1996
In-life End Date	August 20, 1996
Experimental Termination Date	(To be completed when the final report is issued)
Study Completion Date	(To be completed when the final report is issued)

KEY PERSONNEL

Acute Studies

Steven M. Glaza
Study Director
Manager

Steven R. Sorenson
Study Coordinator

Jeffrey B. Hicks
In-life Supervisor

Rose M. Bridge
Administrative Supervisor

Toxicology Support

Kathy Myers
Manager

Calvin L. Horton
Supervisor

Quality Assurance

Sherry R. W. Petsel
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Laboratory Animal Medicine

Cindy J. Cary, DVM
Diplomate, ACLAM
Supervisor

Anatomical Pathology

Thomas E. Palmer, PhD
Anatomical Pathologist

Deborah L. Pirkel/
Jack Serfort
Supervisors
Necropsy

Anne Mosher
Supervisor
Pathology Data

CONTENTS

SUMMARY

The test materials, SS0450.01, SS0451.01, and SS0452.01 (8.76% w/w mixture), were evaluated for their acute oral toxicity potential in male and female mice when administered as a single gavage dose at a level of 5 mL/kg of body weight. No mortality was observed with any test material. Two males treated with SS0450.01 and four males treated with SS0451.01 exhibited hypoactivity on the day of treatment. The remaining animals in these treatment groups appeared normal throughout the study. Clinical signs of toxicity observed in the animals treated with SS0452.01 on the day of treatment included hypoactivity, ataxia, and impaired or loss of righting reflex. All animals in all treatment groups returned to a normal appearance by 2.5 hours after dosing. All animals exhibited body weight gain with the exception of two females treated with SS0450.01, one female treated with SS0451.01, and one female treated with SS0452.01 which exhibited insignificant weight losses during the last week of the study. There were no lesions observed at necropsy.

OBJECTIVE

The objective of this study was to assess the acute toxicity of the test materials when administered as a single oral gavage dose to mice.

All procedural times presented in this report fall within the acceptable ranges as specified in the Wisconsin facility of Corning Hazleton Inc. (CHW) Standard Operating Procedure (SOP).

TEST MATERIALS

Identification

The test materials were identified as SS0450.01, SS0451.01, and SS0452.01 and were all described as clear, colorless liquids

Purity, Stability, and Characterization

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions). Any analysis of the prepared test material mixture for SS0452.01 for concentration, homogeneity/solubility, and stability is the responsibility of the Sponsor (to be addressed by amendment if conducted). Information on the synthesis methods, composition, or other characteristics that define the test materials is on file with the Sponsor.

Storage and Retention

The test materials were stored at room temperature. The following materials were returned to the Sponsor's representative (Joyce A. Tippitt, The Procter & Gamble Company, Sharon Woods Technical Center, 11530 Reed Hartman Highway, Cincinnati, OH 45217) after completion of the in-life phase:

- The reserve sample of each test material
- Any unused portion of each test material
- A 2 mL subsample of the 8.76% w/w mixture of SS0452.01
- The unused portion of the 8.76% w/w mixture of SS0452.01

The reserve samples will be retained by the Sponsor in accordance with 40 CFR 729.

Safety Precautions

The test material handling procedures were according to CHW SOPs and policies.

TEST SYSTEM

Test Animal

Young adult albino mice of the Crl:CD-1®(ICR)BR VAF/Plus® strain were procured from Charles River Laboratories, Inc., Portage, Michigan on July 29, 1996.

Housing

After receipt and during the minimum 7-day acclimation period, the animals were separated by sex and group housed in clear plastic shoebox cages. On the day of study initiation, the animals were transferred to individual housing and remained in the shoebox cages for the duration of the study. Environmental controls for the animal room were set to maintain a temperature of 19° to 25°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark lighting cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to Laboratory Rodent Diet #5001, PMI Feeds, Inc., and water except for approximately 12 to 14 hours before test material administration when food, but not water, was withheld. The feed is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the feed or water at levels that could be expected to interfere with or affect the results of the study.

Animal Selection and Grouping

Fifteen male and 15 female healthy, acclimated mice, exhibiting normal behavior were selected at random for the definitive study. The animals, weighing from 21.5 to 25.8 g, were divided into the following treatment groups:

		Dose Level	Number of Animals	
Group	Test Material	(mL/kg)	Male	Female
1	SS0450.01	5	5	5
2	SS0451.01	5	5	5
3	SS0452.01 (8.76% w/w mixture)	5	5	5

The males were approximately 4 to 6 weeks of age, while the females were approximately 7 weeks of age at initiation of the study. The animals were identified by animal number and corresponding neck tag throughout the study.

Justification for Species Selection

The mouse is frequently used in safety evaluation studies as a representative of a rodent species.

PROCEDURES

Preparation and Administration of Test Materials

An individual dose of the respective test material was calculated for each animal based on its fasted body weight and administered by gavage. SS0450.01 and SS0451.01 were dosed undiluted. SS0452.01 was prepared as a 8.76% w/w mixture in deionized water. Each test material/mixture was administered at a dose level of 5 mL/kg of body weight.

Reason for Route of Administration

A potential route of exposure in humans is oral.

Observations

Observations for mortality and moribundity were conducted twice a day (morning and afternoon) for 13 days after test material administration and again the morning of Day 14.

On the day of dosing, each definitive study animal was observed in its cage. The animals were observed before dosing; continuously for 2 hours after treatment; at approximately 2.5, 3.0, 3.5, 4.0, 6.0, and 8.0 hours postdose; and daily thereafter for 14 days (at approximately the same time each day). The observations included a neurological assessment as described in Appendix 1 of the protocol.

Body weights were determined before test material administration (Day 0), at Day 7, and at termination of the in-life phase (Day 14).

Pathology

At termination of the in-life phase, all animals were euthanized with an overexposure to carbon dioxide and were subjected to a necropsy examination and any abnormalities were recorded. The necropsy included an abbreviated examination of the external surface of the body; all orifices; the cranial cavity; the brain and spinal cord; the nasal cavity and paranasal sinuses; and the thoracic, abdominal and pelvic cavities and viscera. Specific attention was given to the appearance of the stomach and gastrointestinal tract. After necropsy, the animals were discarded and no tissues were saved.

Statistical Analyses

No statistical analyses were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of CHW for a period of 15 years in accordance with CHW SOP.

RESULTS

Mortality

A summary of the survival rate is in Table 1. No mortality was observed during the study.

Body Weights

Individual and mean body weights and body weight gains are in Table 2. All animals exhibited body weight gain with the exception of two females treated with SS0450.01 (Group 1), one female treated with SS0451.01 (Group 2), and one female treated with SS0452.01 (Group 3) which exhibited insignificant weight losses of 0.2 to 1.2 g during the last week of the study.

Clinical Signs

Individual clinical signs are in Table 3. All animals in Groups 1 and 2 appeared normal throughout the study with the exception of two males treated with SS0450.01 and four

males treated with SS0451.01 which were hypoactive within 2.0 hours post-dose. Clinical signs of toxicity observed in the animals treated with SS0452.01 included hypoactivity, ataxia, and impaired or loss of righting reflex. The onset of clinical signs in this group generally occurred within 3 to 5 minutes after dosing. All animals returned to a normal appearance by 2.5 hours after treatment.

Pathology

Individual gross necropsy pathology findings are in Table 4. A summary report by the study pathologist is on Page 15. There were no lesions observed at necropsy.

DISCUSSION

The acute oral toxicity of SS0450.01, SS0451.01, and SS0452.01 (8.76% w/w mixture) was evaluated in male and female mice when administered as a single gavage dose in the definitive study at a level of 5 mL/kg of body weight. No mortality was observed during the study. Two males treated with SS0450.01 and four males treated with SS0451.01 exhibited hypoactivity within 2.0 hours of dosing. The remaining animals in these treatment groups appeared normal throughout the study. Clinical signs of toxicity observed in the animals treated with SS0452.01 on the day of treatment included hypoactivity, ataxia, and impaired or loss of righting reflex. All animals in all groups returned to a normal appearance by 2.5 hours after treatment. All animals exhibited body weight gain throughout the study with the exception of two females treated with SS0450.01, one female treated with SS0451.01, and one female treated with SS0452.01 which exhibited insignificant weight losses during the last week of the study. There were no lesions observed at necropsy.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

Date

PATHOLOGY REPORT

There were 10 mice (five males, five females) each from three groups euthanized and necropsied at the termination of the study. The test material, dose level, day of death, and gross observations recorded for each animal are in the Individual Pathology Comments that follow this report. There were no visible lesions in any of the animals.

Thomas E. Palmer, PhD
Pathologist

Date

(60704511.dft)
091996

Table 1

Mortality Summary

Test Material	Dose Level (mL/kg)	Sex	Mortality Result No. Died/No. Dosed
SS0450.01	5	M	0/5
	5	F	0/5
SS0451.01	5	M	0/5
	5	F	0/5
SS0452.01 (8.76% w/w mixture)	5	M	0/5
	5	F	0/5

Table 2

**Individual and Mean Body Weights/
Body Weight Gains (g) - Definitive Study**

Group 1 - SS0450.01

Animal Number	Day 0 Weight	Day 7		Day 14	
		Weight	Gain*	Weight	Gain*
		Males (5 mL/kg)			
A00840	24.1	31.9	7.8	34.4	10.3
A00841	21.5	28.4	6.9	31.9	10.4
A00842	22.9	30.3	7.4	31.5	8.6
A00838	24.2	31.1	6.9	33.5	9.3
A00839	22.5	30.0	7.5	32.6	10.1
Mean	23.0	30.3	7.3	32.8	9.7
		Females (5 mL/kg)			
A00867	23.3	24.5	1.2	24.9	1.6
A00864	24.1	25.3	1.2	24.5	0.4
A00863	23.3	27.4	4.1	28.4	5.1
A00865	24.0	25.6	1.6	24.9	0.9
A00866	23.9	25.6	1.7	26.7	2.8
Mean	23.7	25.7	2.0	25.9	2.2

* Gain from the Day 0 body weight.

Table 2 (Continued)

Individual and Mean Body Weights/ Body Weight Gains (g) - Definitive Study

Group 2 - SS0451.01

Animal Number	Day 0 Weight	Day 7		Day 14	
		Weight	Gain*	Weight	Gain*
		Males (5 mL/kg)			
A00846	22.8	30.6	7.8	33.0	10.2
A00847	23.3	29.3	6.0	32.9	9.6
A00845	25.8	33.3	7.5	35.7	9.9
A00844	24.6	31.2	6.6	33.8	9.2
A00843	22.6	29.4	6.8	31.8	9.2
Mean	23.8	30.8	6.9	33.4	9.6
		Females (5 mL/kg)			
A00868	23.0	26.0	3.0	27.6	4.6
A00869	23.8	26.6	2.8	27.2	3.4
A00870	23.2	26.6	3.4	27.7	4.5
A00871	23.1	25.9	2.8	26.5	3.4
A00872	23.5	25.1	1.6	23.9	0.4
Mean	23.3	26.0	2.7	26.6	3.3

* Gain from the Day 0 body weight.

Table 2 (Continued)

Individual and Mean Body Weights/ Body Weight Gains (g) - Definitive Study

Group 3 - SS0452.01 (8.76% w/w mixture)

Animal Number	Day 0 Weight	Day 7		Day 14	
		Weight	Gain*	Weight	Gain*
		Males (5 mL/kg)			
A00848	22.8	28.0	5.2	30.5	7.7
A00849	25.0	30.1	5.1	31.7	6.7
A00850	23.6	30.0	6.4	32.8	9.2
A00851	24.6	30.0	5.4	32.5	7.9
A00852	25.1	31.0	5.9	35.3	10.2
Mean	24.2	29.8	5.6	32.6	8.3
		Females (5 mL/kg)			
A00876	23.9	26.3	2.4	27.4	3.5
A00877	23.1	27.1	4.0	26.9	3.8
A00875	23.0	25.5	2.5	25.8	2.8
A00873	24.5	27.6	3.1	28.8	4.3
A00874	23.1	25.6	2.5	26.3	3.2
Mean	23.5	26.4	2.9	27.0	3.5

* Gain from the Day 0 body weight.

Table 3

Individual Clinical Signs - Definitive Study

Group 1 - SS0450.01

Animal Number	Observation	Day 0 Interval: Pre-dose	Hour: 0.0-2.0	2.5	3.0	3.5	4.0	6.0	8.0	Day: 1	2-14
Males (5 mL/kg)											
A00840	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00842	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00839	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00838	Appeared normal	✓	✓(111-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Hypoactivity	-	✓(17-65)	-	-	-	-	-	-	-	-
	Hypoactivity	-	✓(96-111)	-	-	-	-	-	-	-	-
A00841	Appeared normal	✓	✓(69-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Hypoactivity	-	✓8-69	-	-	-	-	-	-	-	-
Females (5 mL/kg)											
A00867	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00864	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00863	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00865	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00866	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.
( ) Superscript numbers in parentheses indicate the start and end times of the observed clinical sign. The times were recorded in minutes postdose.
Comment: Each animal appeared normal from the time of dosing up to the time recorded for the first clinical sign (when applicable).

Table 3 (Continued)

Individual Clinical Signs - Definitive Study

Group 2 - SS0451.01

Animal Number	Observation	Day 0 Interval: Pre-dose	Hour: 0.0-2.0	2.5	3.0	3.5	4.0	6.0	8.0	Day: 1	2-14
Males (5 mL/kg)											
A00846	Appeared normal	✓	-	✓	✓	✓	✓	✓	✓	✓	✓
	Hypoactivity	-	✓(65-120)	-	-	-	-	-	-	-	-
A00847	Appeared normal	✓	-	✓	✓	✓	✓	✓	✓	✓	✓
	Hypoactivity	-	✓(64-120)	-	-	-	-	-	-	-	-
A00845	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00844	Appeared normal	✓	✓(110-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Hypoactivity	-	✓(59-110)	-	-	-	-	-	-	-	-
A00843	Appeared normal	✓	✓(50-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Hypoactivity	-	✓(13-50)	-	-	-	-	-	-	-	-
Females (5 mL/kg)											
A00868	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00869	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00870	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00871	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A00872	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.
( ) Superscript numbers in parentheses indicate the start and end times of the observed clinical sign. The times were recorded in minutes postdose.
Comment: Each animal appeared normal from the time of dosing up to the time recorded for the first clinical sign (when applicable).

Table 3 (Continued)

Individual Clinical Signs - Definitive Study

Group 3 - SS0452.01 (8.76% w/w mixture)

Animal Number	Observation	Pre-dose	Day 0 Interval Hour 0	0-2.0	2.5	3.0	3.5	4.0	6.0	8.0	Day 1	Day 2-14
			Males (5 mL/kg)									
A00848	Appeared normal	✓	✓(16-120)	✓	✓	✓	✓	✓	✓	✓	✓	
	Ataxia	-	✓(6-16)	-	-	-	-	-	-	-	-	
	Impaired righting reflex	-	✓(6-10)	-	-	-	-	-	-	-	-	
	Hypoactivity	-	✓(6-16)	-	-	-	-	-	-	-	-	
A00849	Appeared normal	✓	✓(15-120)	✓	✓	✓	✓	✓	✓	✓	✓	
	Hypoactivity	-	✓(5-15)	-	-	-	-	-	-	-	-	
	Ataxia	-	✓(5-15)	-	-	-	-	-	-	-	-	
A00850	Appeared normal	✓	✓(36-120)	✓	✓	✓	✓	✓	✓	✓	✓	
	Ataxia	-	✓(4-20)	-	-	-	-	-	-	-	-	
	Loss of righting reflex	-	✓(4-8)	-	-	-	-	-	-	-	-	
	Hypoactivity	-	✓(4-36)	-	-	-	-	-	-	-	-	
A00851	Appeared normal	✓	✓(37-120)	✓	✓	✓	✓	✓	✓	✓	✓	
	Ataxia	-	✓(4-20)	-	-	-	-	-	-	-	-	
	Loss of righting reflex	-	✓(4-15)	-	-	-	-	-	-	-	-	
	Hypoactivity	-	✓(4-37)	-	-	-	-	-	-	-	-	
A00852	Appeared normal	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	
	Ataxia	-	✓(3-18)	-	-	-	-	-	-	-	-	
	Hypoactivity	-	✓(3-120)	-	-	-	-	-	-	-	-	

✓ Condition existed.

- Condition not evident.

( ) Superscript numbers in parentheses indicate the start and end times of the observed clinical sign. The times were recorded in minutes postdose.

Comment: Each animal appeared normal from the time of dosing up to the time recorded for the first clinical sign (when applicable).

Table 3 (Continued)

Individual Clinical Signs - Definitive Study

Group 3 - SS0452.01 (8.76% w/w mixture)

Animal Number	Observation	Day 0 Interval: Pre-dose	Hour: 0.0-2.0	2.5	3.0	3.5	4.0	6.0	8.0	Day: 1	2-14
			Females (5 mL/kg)								
A00876	Appeared normal	✓	✓(14-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Ataxia	-	✓(4-14)	-	-	-	-	-	-	-	-
A00877	Appeared normal	✓	✓(12-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Ataxia	-	✓(3-12)	-	-	-	-	-	-	-	-
	Hypoactivity	-	✓(3-12)	-	-	-	-	-	-	-	-
	Impaired righting reflex	-	✓(3-12)	-	-	-	-	-	-	-	-
A00875	Appeared normal	✓	✓(33-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Ataxia	-	✓(19-27)	-	-	-	-	-	-	-	-
	Impaired righting reflex	-	✓(19-23)	-	-	-	-	-	-	-	-
	Hypoactivity	-	✓(19-33)	-	-	-	-	-	-	-	-
A00873	Appeared normal	✓	✓(32-120)	✓	✓	✓	✓	✓	✓	✓	✓
	Ataxia	-	✓(3-11)	-	-	-	-	-	-	-	-
	Hypoactivity	-	✓(4-32)	-	-	-	-	-	-	-	-
A00874	Appeared normal	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.
- Condition not evident.
( ) Superscript numbers in parentheses indicate the start and end times of the observed clinical sign. The times were recorded in minutes postdose.

Comment: Each animal appeared normal from the time of dosing up to the time recorded for the first clinical sign (when applicable).

Table 4

Individual Pathology Comments

Group 1 - SS0450.01
Dose Level: 5 mL/kg of Body Weight

Animal Number	Sex	Test Day		Necropsy Observation
		Died	Sacrificed	
A00840	M	-	14	No visible lesions.
A00841	M	-	14	No visible lesions.
A00842	M	-	14	No visible lesions.
A00838	M	-	14	No visible lesions.
A00839	M	-	14	No visible lesions.
A00867	F	-	14	No visible lesions.
A00864	F	-	14	No visible lesions.
A00863	F	-	14	No visible lesions.
A00865	F	-	14	No visible lesions.
A00866	F	-	14	No visible lesions.

- Not applicable.

Table 4 (Continued)

Individual Pathology Comments

Group 2 - SS0451.01
Dose Level: 5 mL/kg of Body Weight

Animal Number	Sex	Test Day		Necropsy Observation
		Died	Sacrificed	
A00846	M	-	14	No visible lesions.
A00847	M	-	14	No visible lesions.
A00845	M	-	14	No visible lesions.
A00844	M	-	14	No visible lesions.
A00843	M	-	14	No visible lesions.
A00868	F	-	14	No visible lesions.
A00869	F	-	14	No visible lesions.
A00870	F	-	14	No visible lesions.
A00871	F	-	14	No visible lesions.
A00872	F	-	14	No visible lesions.

- Not applicable.

Table 4 (Continued)

Individual Pathology Comments

Group 3 - SS0452.01
Dose Level: 5 mL/kg of Body Weight

Animal Number	Sex	Test Day		Necropsy Observation
		Died	Sacrificed	
A00848	M	-	14	No visible lesions.
A00849	M	-	14	No visible lesions.
A00850	M	-	14	No visible lesions.
A00851	M	-	14	No visible lesions.
A00852	M	-	14	No visible lesions.
A00876	F	-	14	No visible lesions.
A00877	F	-	14	No visible lesions.
A00875	F	-	14	No visible lesions.
A00873	F	-	14	No visible lesions.
A00874	F	-	14	No visible lesions.

- Not applicable.

APPENDIX

Protocol Deviation
Protocol
Protocol Amendment No. 1

(The contents of this appendix will be
page numbered when the final report is issued)

Protocol Deviation

Protocol	Actual Procedure
Page 7, 6. Experimental Design, C. Dosing Procedures, (4) Dose Preparation. Second sentence. Test substance SS0452.01 will be diluted to a 8.75% w/w concentration with deionized water and this mixture administered at a dose volume of 5.0 mL/kg.	While preparing the dilution of SS0452.01, excess deionized water was inadvertently added to the beaker which contained test material. An appropriate amount of additional test material was added to the mixture to achieve the desired concentration. After the dilution of SS0452.01, the final concentration was calculated to be 8.76%.

This deviation is not considered to have had an adverse effect on the outcome of the study.

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CORNING Hazleton

Sponsor:

The Procter & Gamble Company
Cincinnati, Ohio

PROTOCOL TP6522

Study Title:

Acute Oral Gavage Toxicity Study with SS0450.01, SS0451.01 and SS0452.01 In Mice with Additional Neurological Examinations

Date:

July 30, 1996

Performing Laboratory:

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

Laboratory Project Identification:

CHW 60704511

Sponsor Project Identification:

SSBTS-96.010

STUDY IDENTIFICATION

Acute Oral Gavage Toxicity Study with SS0450.01, SS0451.01 and SS0452.01 In Mice with Additional Neurological Examinations

Test Material Identification Numbers	SS0450.01, SS0451.01 and SS0452.01
Sponsor	The Procter & Gamble Company Sharon Woods Technical Center 11530 Reed Hartman Highway Cincinnati, OH 45217
Study Monitor	Cathy Satter, PhD The Procter & Gamble Company Sharon Woods Technical Center 11530 Reed Hartman Highway Cincinnati, Ohio 45217 (513) 626-3458 Facsimile No. (513) 626-3522
Study Director	Steven M. Glaza Corning Hazleton Inc. P.O. Box 7545 Madison, Wisconsin 53707 (608) 241-7292 Facsimile No. (608) 242-7936
Study Location	Corning Hazleton Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704
Proposed Study Timetable	
Experimental Start Date	August 6, 1996
Experimental Termination Date	August 20, 1996

1. Study
   Acute Oral Gavage Toxicity Study with SS0450.01, SS0451.01 and SS0452.01 in Mice with Additional Neurological Examinations

2. Purpose
   To assess the acute toxicity of the test materials when administered as a single oral gavage dose to mice

3. Regulatory Compliance
   This study will be conducted in accordance with the United States Environmental Protection Agency's Good Laboratory Practice Standards, 40 CFR 792 with the exception that analysis of the prepared test material mixture for SS0452.01 for concentration, homogeneity/solubility, and stability will not be conducted.

4. Quality Assurance
   The protocol, study conduct, and final report will be audited by the Quality Assurance Unit in accordance with the Standard Operating Procedures (SOPs) of the Wisconsin facility of Corning Hazleton Inc. (CHW).

5. Test Materials

   A. Test Materials

      (1) Identification
      SS0450.01, SS0451.01 and SS0452.01

      (2) Purity
      Purity information (including under test conditions) is the responsibility of the Sponsor.

      (3) Stability
      Stability information (including under test conditions) is the responsibility of the Sponsor

      (4) Storage Conditions
      To be provided by the Sponsor

      (5) Characteristics
      Information on synthesis methods, composition, or other characteristics that define the test materials is on file with the Sponsor.

B. Reserve Samples
A reserve sample (approximately 2 mL or 2 g) of each lot of test material will be taken on the day of treatment and stored at room temperature. These samples will be transferred to the Sponsor after completion of the in-life phase to be retained in accordance with 40 CFR 792.

C. Disposition of Test Material
Any remaining test material will be returned after authorization from the Sponsor. Test material will be sent to:

Joyce A. Tippitt
The Procter & Gamble Company
Sharon Woods Technical Center
11530 Reed Hartman Highway
Cincinnati, OH 45217

D. Safety Precautions
As required by CHW SOPs, unless otherwise specified

6. Experimental Design

A. Animals

(1) Species
Mouse

(2) Strain
Crl:CD-1®(ICR)BR VAF/Plus®

(3) Source
Charles River Laboratories, Inc.

(4) Age at Initiation of Treatment
Approximately 4 to 6 weeks

(5) Weight at Initiation of Treatment
20 to 35 g

(6) Number and Sex
5 males and 5 females for each dose level/test material

(7) Identification
Individually numbered neck tags

(8) Husbandry

(a) Housing
Individual (may be group-housed during acclimation) in clear plastic shoebox cages

(b) Food
Laboratory Rodent Diet® #5001 (PMI® Feeds, Inc.) *ad libitum*, unless otherwise specified. The food is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

(c) Water
Ad libitum from water bottles. Samples of the water are analyzed for total dissolved solids and specified microbiological content and for selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons.

(d) Contaminants
There are no known contaminants in the food or water that would interfere with this study

(e) Environment
Environmental controls for the animal room will be set to maintain 19° to 25°C (66° to 77°F), a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

(f) Acclimation
At least 1 week

(9) Selection of Animals
Animals meeting health and body weight requirements, and exhibiting normal behavior, will be selected at random for each dose level.

(10) Justification for Species Selection
The mouse is frequently used in safety evaluation studies as a representative of a rodent species.

B. Study Design

The animals will be assigned to groups as follows:

Group	Test Material	Dose Level (mL/kg)[a]	Number of Animals Male	Female
1	SS0450.01	5	5	5
2	SS0451.01	5	5	5
3	SS0452.01	5	5	5

a Additional dose levels consisting of 5 males and 5 females may be added based on the results of the initial dose levels. These dose levels will be varied using a multiplier of 1.3 following an "up/down" study procedure. Justification for the additional dose levels will be provided by protocol amendment.

C. Dosing Procedures

(1) Dosing Route
Oral gavage

(2) Reason for Dosing Route
A potential route of exposure in humans is oral.

(3) Dosing Duration
The animals will be fasted (food withheld) overnight (approximately 12 - 14 hours). After the fasting period, the animals will be weighed and the respective test material administered orally as a single dose. Individual dose volumes will be calculated based on the animal's fasted body weight. Animals will be returned to *ad libitum* feeding approximately

2 hours after dose administration. The day of treatment will be designated as Day 0.

(4) Dose Preparation
SS0450.01 and SS0451.01 will be dosed as received (undiluted). Test substance SS0452.01 will be diluted to a 8.75% w/w concentration with deionized water and this mixture administered at a dose volume of 5.0 mL/kg. A 2 mL subsample of this test mixture will be returned to the Sponsor's representative listed in section 5.C. under ambient conditions on the day of treatment. Any analysis of this mixture will be addressed by amendment. Dose levels will be varied by changing the dose volume. The test materials and prepared test mixture will be stored at room temperature prior to dosing.

D. Observation of Animals

(1) Antemortem Observations
Twice daily (a.m. and p.m.) for mortality and moribundity. Additional signs of poor health or abnormal behavior will be recorded as they are observed.

(2) Predose and Postdose Observations
Each animal will be observed predose (1-15 minutes prior to dosing), continuously for 2 hours, approximately 2.5, 3.0, 3.5, 4.0, 6.0, and 8.0 hours postdose; and daily thereafter for 14 days (approximately the same time each day). The observations will include specific evaluation of ataxia and righting reaction. The procedures for these neurological assessments are described in Appendix 1.

(3) Body Weights
On the first day of treatment and weekly thereafter. Body weights will also be recorded for animals sacrificed at an unscheduled interval or when found dead.

E. Termination

(1) Unscheduled Sacrifices and Deaths
Abbreviated necropsies will be done. Animals to be sacrificed will be euthanized with an overexposure to carbon dioxide.

(2) Scheduled Sacrifice
On Day 14, surviving animals will be euthanized and necropsied.

F. Postmortem Procedures

(1) Necropsy
The necropsy will include an abbreviated examination of:

External surface of the body
All orifices
Cranial cavity
Brain and spinal cord
Nasal cavity and paranasal sinuses
Thoracic, abdominal, and pelvic cavities and viscera
Specific attention will be made to the stomach and gastrointestinal tract

(2) Tissue Preservation
Will not be done

(3) Histopathology
Will not be done

7. Reports
A final report including, but not limited to, those items listed below will be submitted. One copy of the audited draft report and two copies of the final report will be provided.

A. Experimental Design and Methods
As defined by the protocol and any protocol amendments and protocol deviations

B. Results
Mortality (to include fate and date of death for each animal)
Antemortem observations
Body weights
Body weight gains
Macroscopic observations

C. Statistical Analyses
No statistical analysis of the data will be required.

D. Compliance Statements
A GLP compliance statement signed by the study director and a Quality Assurance statement will be provided.

8. Maintenance of Raw Data and Records
Original data or copies will be available at CHW to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, original paper data including those items listed below will be retained in the archives of CHW for a period of 15 years following signing of the final report. Fifteen years after the signing of the final report, all of the aforementioned materials will be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the CHW archives for additional time, and a storage fee will be charged. If the Sponsor chooses to have CHW dispose of the materials, a disposal fee will be charged.

Protocol and protocol amendments
Dose preparation records
In-life records
  Animal room maintenance
  Dose administration
  Antemortem observations
  Body weights
Anatomical pathology records
Study correspondence
Final report (original signed copy)

The following supporting records will be retained at CHW but will not be archived with the study data.

Water analysis records
Animal room temperature and humidity records
Refrigerator and freezer temperature records
Instrument calibration and maintenance records
Animal receipt/acclimation records

9. Protocol Amendments

Alterations to this protocol may be made as the study progresses. No changes in the protocol will be made without the specific consent of the Sponsor's Representative or Monitor. Each protocol amendment will be prepared and signed by the Study Director, Sponsor's Representative or Monitor, and Quality Assurance Unit for any such changes.

PROTOCOL APPROVAL

Cathy S. Satter

Cathy Satter, PhD
Study Monitor
The Procter & Gamble Company

7-31-96
Date

Steven M. Glaza

Steven M. Glaza
Study Director
Acute Studies
Corning Hazleton Inc.

7-30-96
Date

Randy Lesty

Representative
Quality Assurance Unit
Corning Hazleton Inc.

7.30.96
Date

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APPENDIX 1

Neurological Assessment

The following neurological assessments will be made prior to dosing (approximately 1-15 minutes prior to dosing), continuously for 2 hours, approximately 2.5, 3.0, 3.5, 4.0, 6.0 and 8.0 hours postdose. These neurological assessments will also be made daily thereafter for 14 days. Daily evaluations will be conducted at the approximate same time each day during the 14 day observation period.

All neurological observations will be conducted while the animal is in it's individual plastic shoebox cage.

Ataxia: Ataxia is defined by reeling, lurching or staggering gait. Ataxia will be evaluated by observing spontaneous locomotor activity. Each animal will be prompted to walk, if necessary. If ataxia is observed, the time of onset and duration will be recorded.

Righting Reaction: Animals exhibiting abnormal behavior will be tested for the righting reaction. Righting reaction will be assessed by placing the animal on its back. If loss of righting reaction is observed, the time of onset and duration will be recorded.

Other: Tremors, convulsions or other signs indicative of neurotoxicity will also be recorded. These additional signs will be documented by the time and onset and duration.

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CORNING Hazleton

AMENDMENT NO. 1 TO THE PROTOCOL

PROTOCOL TP6522

Acute Oral Gavage Toxicity Study with SS0450.01, SS0451.01 and SS0452.01 In Mice with Additional Neurological Examinations

CHW 60704511

Sponsor Project Identification:

SSBTS-96.010

Sponsor

The Procter & Gamble Company
Sharon Woods Technical Center
11530 Reed Hartman Highway
Cincinnati, OH 45217

Sponsor's Representative

Cathy Satter, PhD

Testing Facility

Corning Hazleton Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Study Director

Steven M. Glaza

This amendment modifies the following portions of the protocol:

Effective July 30, 1996

1. **Page 4, 5. Test Materials, B. Reserve Samples.** Due to the limited amount of the SS0452.01 test material received, a 2 mL reserve sample will not be required. Substitute the following for the first sentence of this section:

A 2 mL reserve sample of the SS0450.01 and SS0451.01 test materials will be taken. Any remaining SS0452.01 test material left after dose preparation will be considered the reserve sample. These samples will be taken on the day of treatment and stored at room temperature.

2. **Page 4, 6. Experimental Design; A. Animals; (4) Age at Initiation of Treatment.** To accommodate the use of the animals that were available for testing, replace this section with the following

Males	Approximately 4 to 6 weeks
Females	Approximately 4 to 8 weeks

PROTOCOL AMENDMENT APPROVAL

Cathy Satter, PhD
Sponsor's Representative
The Procter & Gamble Company

Date

Steven M. Giaza
Study Director
Acute Studies
Corning Hazleton Inc.

9-18-96
Date

Representative
Quality Assurance Unit
Corning Hazleton Inc.

9.18.96
Date